REGENERATION IN PLANTS. I.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXVI.

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(WITH FOURTEEN FIGURES)

INTRODUCTION.

THE term regeneration has come to be used by most botanical writers with a broad and somewhat indefinite application.   Its essential feature, however, is the replacement of an organ or structure that has been removed.   This is accomplished in a variety of ways. PRANTL (9) first found and later SIMONS (11) determined more accurately that if the tip of a root be cut off not more than 0.75^{mm} from the end there is a complete restoration of the part removed, a new tip forming out of the tissues at the cut surface.   GOEBEL (3, p. 503) has shown that if the young apex of the frond of Polypodium be cut in two lengthwise, the remaining embryonic tissue on each piece will completely reform the half that has been removed.   The same is true of the growing point of a fern prothallium, although the older parts are not replaced.   These phenomena are quite homologous with regeneration as it occurs in animals.   If we cut off the root tip somewhat farther back, however, a new tip is not organized at the cut surface, but behind it one or perhaps more new root primordia are organized, and these take the place of the main root.   Or if we cut off transversely a portion of the thallus of Marchantia or Lunularia (12), the tissues at the cut surface will not develop, but there will arise from apparently mature and differentiated cells back of the cut new outgrowths of thallus which again will complete the plant.

If the shoot with all the buds be severed from the root of Taraxacum, new shoots will arise lower down from the mature tissues of the cortex.   Many fleshy roots have this capacity, and if cut into a number of pieces each will organize new primordia and develop shoots.   If the young stem of Convolvulus, Linaria, and other plants (6) be cut off just below the cotyledons, there will arise on the sur-

face of the hypocotyl outgrowths which develop into new shoots. These shoots also arise from mature cells which in the normal course of events remain as permanent tissue.   Nor is this power of organizing new shoot primordia confined to the stems and roots, but is also possessed by many leaves, as in the well known cases of Begonia,
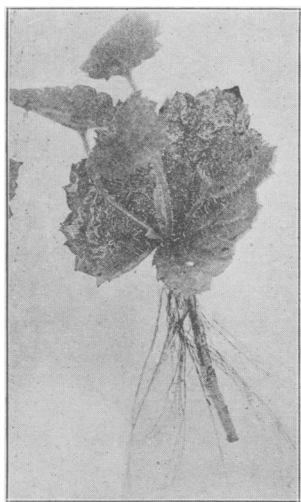

FIG. 1

Bryophyllum, *Cardamine pratensis, Tolmiea Menziesii*, and many other plants (*fig. 1*).   Many stems, probably the majority, if removed from the root system and kept moist will produce new roots.   In a few cases, as in Salix, there may exist on the stem primordia already organized, but in the great majority of stems these are not present.   If a portion of the stem of Salix be cut out from the rest and kept moist, there will appear on it both roots and shoots, each arising, however, from buds already laid down. In the axils of the leaves of many annual shoots are very minute bud primordia, which normally do not develop.   If the top of the plant be cut off, these at once form new shoots.   In our trees and shrubs the buds formed in the leaf axils do not develop until the following year; but if at any time during the spring the tip of the young shoot be removed, a number of these buds, usually those near the top, at once develop shoots.

We have in these cases at least three seemingly diverse phenomena: (1) the part removed is entirely restored by the growth of the cells immediately at the cut surface; (2) there is no growth of embryonic tissue at the wounded surface, but at a greater or less distance from it the organization of entirely new primordia which develop organs that replace those removed; (3) the organ removed, *e. g.*, the shoot, is restored by the development of already existing dormant buds. Between these no hard and fast lines can be drawn, for they all exhibit intergradations, and between the third case—the development of latent buds—and normal vegetative growth no sharp separation can

be made, for occasionally in some species, e. g., Salix, the axillary buds on the first year's growth instead of remaining dormant until the following spring will develop at once into shoots.

It will be quite apparent that as regeneration merges so insensibly into ordinary vegetative growth, the necessary limitations as to the use of the term must be entirely artificial. PFEFFER (8) restricts the term to those cases where an organ directly replaces that portion of itself that has been removed; all others he would call mere reproduction. GOEBEL, KLEBS, MORGAN, KÜSTER, and most other writers on the subject, give it a broader meaning, so as to include the replacement of parts or organs, whether by means of entirely new growths, or from the development of latent buds. The advantage in having some general expression to cover all these phenomena, and the fact mentioned by MORGAN, that they all accomplish the same result and are probably due to the same cause, make it a matter of convenience to use the term in its wider application.

A certain amount of confusion has arisen because it has not been kept clear that regeneration is not really different from ordinary vegetative growth. Most plants naturally tend to grow and branch indefinitely, the new members arising usually in definite places, the shoot primordia, for example, in the embryonic parts of the shoot, and the root primordia ordinarily in the younger regions of the root. The fact that this is the general rule has led to an unjustifiably rigid limitation of the origin of new members to specified regions. As a matter of fact, the ability to produce new members is distributed throughout the plant body, and in many even of the higher plants almost any part is able to produce any other vegetative part. Nor is this ability limited to embryonic parts, for in very many plants it is exercised by the older cells, as in the production of shoots on roots of Taraxacum or on leaves of Begonia. That certain conditions are necessary to bring this latent ability into activity does not make it in the least different from ordinary vegetative growth, for the latter also is dependent on definite conditions.

The whole plant body of mosses and liverworts, and many roots, stems, and leaves of the vascular plants have this capacity, and it requires only the proper conditions to become manifest.

In spite of the extensive investigations into this question, ranging

as they have throughout the greater part of the plant kingdom, our knowledge of what these conditions are is very obscure. We know little enough of the external factors concerned and almost nothing at all definite about the internal ones. When a part of the plant body is removed, many factors are necessarily disturbed. The nutritive conditions may be profoundly altered, as also may be the water relation. The influence of the wound itself may be important, and independent of these the mere absence of the organ may in itself be of far reaching influence. How far any of these may be responsible for regeneration is not clear. Various theories have been proposed, but none have as yet been supported by adequate experimental evidence.
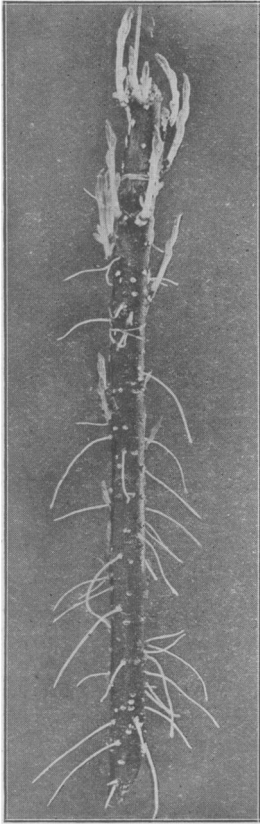
Intimately associated with this problem of regeneration is that of polarity, for almost invariably the new structures occur in such a manner as to exhibit this remarkable phenomenon (*fig. 2*), and if we can determine the exact cause of the appearance of roots or shoots in an isolated piece of Salix stem, for example, the reason for their development at certain places only may be apparent. At present we are blocked at the outset by not knowing, at least under most circumstances, the stimulus which incites their development at all.

In conducting some investigations in this subject it soon became evident that the best method of attack would be to take all the possible factors and work on them separately, subjecting each, one at a time, to a more exact physiological analysis. The effort was made to determine whether the proposed cause in any given case of regeneration is a necessary part of the stimulus, by endeavoring to devise conditions under which the regeneration could be induced to occur in its absence. By this process of

FIG. 2

exclusion the essential factor might be isolated.   Experiments were conducted on Phaseolus, Salix, Helianthus, Taraxacum, Tolmiea, and other plants.

If the common scarlet runner bean, a variety of *Phaseolus mul-tiflorus*, be cut off any place along the epicotyl, there arise from within the cotyledons two shoots, which grow vigorously and may attain the size of the normal plants (*fig. 3*).   Sometimes, however, one of these may grow weakly, or even be entirely suppressed.   These arise from two minute primordia which are present, one in the axil of each cotyledon.   Of the many hundred plants under observation scarcely a case was seen in which these primordia developed without the removal of the shoot, and in every case in which the stem was cut off they developed.   Though less than a millimeter in length, in three or four days after the stem is removed they appear above the cotyledons, and in a week are often 6^{cm} long.   Growth is then very rapid, and in a month they may be 60 or 70^{cm} high.   In the axils of each of the foliage leaves on the plant there is, as usual, a bud. These under the con-dition of my experiments rarely developed. If the plant be allowed to grow until the second internode is formed, and this be cut off, these dormant buds in the axils of the leaves at its base will at once become active and give rise to two shoots; or if the plant form several internodes and the upper one be removed, the buds of the nodes below (not necessarily the first one) at once will develop shoots.

FIG. 3

Here we have one of the most common phenomena in regeneration, namely the removal of a part stimulating to development what would otherwise be dormant primordia.   But this same removal or isolation of a part is followed by the growth of organs where their primordia
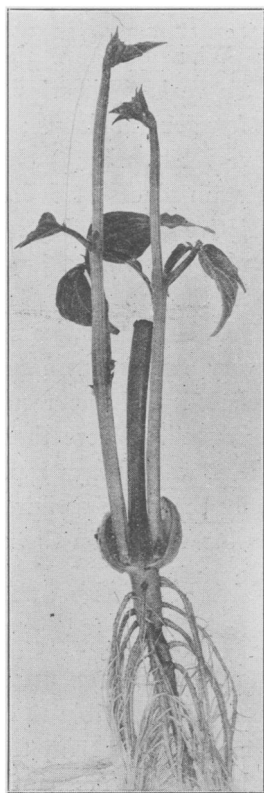
do not exist; for if the stem of the bean be severed from the root system and kept moist, new roots appear along the stem. Similarly, when many roots and leaves or other parts are cut away, new shoot primordia are organized from cortical or other tissues, and it seems quite probable that the same stimulus which starts the development of many latent primordia will in many cases where they are absent incite their origin from already differentiated tissues.

What now are the possible factors operating in these cases? The various theories and possibilities suggested fall naturally into a few general classes: (1) wound stimulus; (2) disturbance in nutritive relations; (3) changes in water content; (4) accumulation at certain places of definite formative substances; (5) correlation; (6) relative age and degree of maturity of the different parts of a member; and (7) growth tensions. Each of these will be discussed in connection with the experiments relating to them.

Unless otherwise stated, the plant used was Phaseolus, and in every experiment a sufficient number of plants were used to insure reliability of the result, and check experiments were always carefully arranged. Where there was any diversity in the result the experiment was always repeated. Of the total number of experiments only the more striking ones will be described, and for convenience in the discussion these will be numbered in the order in which they are presented.

DISTURBANCE IN NUTRITION.

When a growing part is removed, a large part of the food that would have been used by it may now be unused in the plant, and may be accessible to other parts. If during the spring the tip of a shoot of almost any tree be removed, some of the buds which would otherwise lie dormant until the next year develop into shoots (*figs. 6* and *7*). Here, and in Onoclea where GOEBEL (2) secured the metamorphosis of sporophylls into foliage leaves by the continued removal of the former, KLEBS (5) sees an essential factor in the disturbance occurring in the nutritive conditions. In the axils of the cotyledons of *Juglans regia* there are a number of buds which, as GOEBEL (4, p. 209) has pointed out, do not develop unless the terminal bud be removed. This arrest of these buds is due GOEBEL says "to all the available food material being devoted to the devel-

opment of one terminal bud." Many tuber- and bulb-forming plants do not normally produce seeds, but, as in *Lilium candidum, Lachenalia luteola,* etc., if the bulbs be cut away and prevented from forming, seeds will be produced. "In the normal condition," GOE-BEL says, "seed formation is hindered because the plastic material which might be used for seeds streams into the bulb." The inference is that this material, prevented from going to the bulbs, will flow to the seeds. This conception plays a fundamental part in GOEBEL'S explanation of how the removal of one part may start the development of another. SACHS (**10**) gives a similar explanation for the development of the cotyledonary buds in Phaseolus, stating that it is due to increase of food resulting from the removal of the main axis. To demonstrate this it must be shown that these lateral buds, though in intimate contact with large cotyledons, remain undeveloped because of lack of food, and further that when the terminal bud is removed they will not develop if the possibility of an increased amount of food is precluded.

FIG. 4

The attempt is made in the following experiments to determine this experimentally. The plants used, unless otherwise stated, were seedlings, the epicotyl varying from 1 to 9^{cm} long, with the first pair of leaves not yet fully opened, and the cotyledons still full of food (*fig. 4*).

If an increase of nutritive material in the axial primordia, due to the removal of the parts using this while the source of supply is still active, is the cause of development of these structures, then while removing the central shoot, if we at the same time remove the source of the food, there should be no development of the primordia.

Experiment 1.—Twelve plants were used, the epicotyl was cut

off about the middle and the cotyledons left intact.   The axial buds developed in all (*fig. 3*).   This was repeated later for different purposes on scores of plants, and the result was always the same, except that occasionally only one shoot would develop.

Experiment 2.—Twelve plants were used, the epicotyl was cut off, and one cotyledon was removed.   In all cases shoots developed from both axils.

Experiment 3.—Seven plants were used, the epicotyl was cut off, and both cotyledons were cut off but left in their natural position so as not to introduce other factors, as light, air, or moisture from the soil.   All developed shoots from both axils.   This was repeated on many plants with similar results.

Experiment 4.—Three plants, almost mature, 40–50^{cm} high and with 6–8 internodes, were used.   The cotyledons had been used up and had dropped away; the lower part of the stem had become quite hard, almost woody, and hollow.   The plants were cut off a few centimeters above the ground, and five of the six primordia developed shoots.

Here as in the other three experiments it is clear that food derived from the cotyledons is no essential part of the stimulus which causes the young buds to develop.   In the next three experiments the attempt was made to remove the terminal bud and at the same time diminish the nutritive supply of the other buds even to the point of starvation.

Experiment 5.—Five plants that had been grown with their roots in tap water were transferred to distilled water.   All the cotyledons were cut off and also the epicotyl.   All five of the plants slowly but completely regenerated.   Here all supply of food from the substratum, from the cotyledons, and from the leaves is removed.   In this experiment and others a large number of check plants showed that the cutting away of the cotyledons had no effect on the young buds if the primary axis was left intact.

Experiment 6.—Four seeds just germinating were taken, the radicle being 4^{cm} long, and the young stem with its leaves being still between the cotyledons.   The cotyledons were forced open and both cut off, leaving only the small stem and root.   The plants were then placed with the root in distilled water in the dark, and left for two

days.   Both stem and root elongated rapidly, at the expense of the
food in the young plant to start with, so that part, probably most, of
the food was used up, and the rest was distributed throughout the
now much larger plant.   Then the stem was cut off as close as possi-
ble to the buds, and two of the plants were placed in the dark and two
in the light.   All four regenerated slowly.

   Experiment 7.—Twelve young plants were used as follows: *a*,
two were cut off between the point of attachment of the cotyledons
and the buds in their axils, so as to cut away the cotyledons and
hypocotyl, leaving epicotyl with apex intact and primordia at the
base, and set in a moist chamber; *b*, from ten plants the cotyledons
were removed and the stem cut off close above the primordia, then
the hypocotyl was cut off immediately below the primordia, leaving a
small piece of the stem (average 8^{mm} in length) with the primordia
attached; five of these were set in darkness and five in light in a moist
chamber, with the base of each piece resting on wet filter paper.   In
a there was no development of the buds; in *b*, of the buds in the dark
those on four plants grew to be 1–2^{cm} long and then died, presumably
from starvation; while those in the light developed slowly at first
and faster as they formed chlorophyll, finally forming shoots.

   These experiments show conclusively that when the young shoot
is removed the removal also of the food supply does not hinder the
development of the buds at the base, and that it occurs when there
is not only no increase in the food, but when the primordia and the
surrounding parts are in a condition of starvation.   Plants were
grown in the most favorable conditions of rich soil, inorganic nutrient
solutions, light, and moisture, so that vegetative growth was luxuriant,
but only the removal of the apex had any influence in inciting the
buds below to growth.   The constant factor in every case is the removal
of the apex, and neither an increase in food dependent on the removal
of the growing shoot, nor any decrease that might occur in the nutri-
tive relations constitute an essential part of the stimulus.

   In Bryophyllum GOEBEL (**3**, p. 420) says that the vegetative
points serve as "centers of attraction for the constructive materials."
Those on the shoot, according to him, because of their more direct
connections with the conducting system act as stronger attractive
centers than those on the leaves.   Of the buds on the shoot the

terminal one exerts a stronger attraction than the lateral ones. If we cut off the terminal bud the lateral ones develop, and GOEBEL (3, p. 418) has shown that if we cut off all the shoot buds those on the leaves develop. His explanation is that the stronger points of attraction being no longer active, the "building material" is drawn toward the points of "weaker attraction," *i. e.*, the growing points of the leaves.

Such a conception is scarcely in harmony with the principles that control the movement of materials in plants. Any substance in solution in the plant necessarily follows the general laws of solutions, and will diffuse toward any point only when there is less of it there than at the place from which it moves. A movement of constructive materials from the leaves or cotyledons only occurs when there is more of it in solution there than elsewhere, and if the flow is directed toward the terminal bud it is only because that is the point of least concentration. If at any time there were less in the lateral buds than in the terminal one, it would diffuse into the former. The fact of any food material moving toward the terminal part and past the lateral buds is positive evidence that the terminal bud contains less of this in solution (and it is only in solution that it would be available) than the others. There is no evidence for assuming that the food is "attracted" into certain buds in sufficient quantity to start growth and not into others; for until growth starts all will contain an equal amount, and afterwards, if food continues to move toward certain buds and not toward others it can only be because growth (or some other cause) keeps the amount in the former constantly less than in the latter.

Thus there is no ground for the supposition that the buds that develop in regeneration are any better supplied with food after they start to sprout than before, and that when they do not develop, as GOEBEL'S hypothesis implies, it is because of lack of food. Frequently they are stored with food and will begin to develop so soon as isolated from the parent plant. Starvation, as we know, will not cause growth to cease until it is far more severe than it is ever likely to be on any well-nourished plant; and, as MORGAN (7, p. 27) has pointed out, animals regenerate even while starving to death.

Experiment 8.—From young shoots of Salix and Lycium the ter-

minal bud and all the leaves were removed, and the shoots, separated from the parent plants, were placed in the dark. The axillary buds started to develop and continued until all the nourishment in the shoots were exhausted.

Roots of Taraxacum were cut into several pieces, and on each piece new shoots arose. We cannot say that each piece was either better or more poorly nourished than before. A Marchantia thallus grown in very weak light and plainly in a semi-starved condition regenerated when cut across transversely.

It seems clear from the experimental evidence that, at least in the plants mentioned, there need be no increase in nutritive conditions to occasion regeneration, and we must look elsewhere for the stimulus.

DISTURBANCE IN WATER CONTENT.

When the leaves or other transpiring surfaces of a plant are removed, there is often opportunity for a better supply of water in the remaining parts. The profound influence of water as a factor in growth is too well known to need emphasis. If the leaves of many trees or shrubs be removed early enough, the axillary buds will develop shoots instead of remaining dormant until the next year. DeCandolle (1) attributed this to the "sap" being no longer drawn away from the buds by the leaves. Wiesner (15) thinks that the young buds are hindered from developing because the water is withdrawn from them by the more actively transpiring leaves. In isolated pieces of Salix stems Vöchting (13) believes that water is the factor that determines the appearance of roots, and Klebs (5, p. 104) had experimentally proved this to be true in at least some species. Wakker (14) obtained buds on leaves of Bryophyllum by submerging them, and thinks it due to disturbances in the water current. Goebel (3, p. 393) obtained buds on leaves of Aneimia rotundifolia by either submerging them or placing them in very moist air; also on uninjured leaves of Cardamine pratensis by keeping the plants in moist air. Klebs attributes this to the checking of transpiration and consequent abundance of water in the leaf. By surrounding a portion of an uninjured stem of Salix by a glass cylinder filled with water, Klebs (5, p. 104) found that roots develop upon that part. Other plants, especially those whose natural habitat is in wet places,

possess this ability to produce roots along portions of the stem that are kept wet. Sometimes, as in *Veronica anagallis*, the root primordia are already laid down, but in most plants these are not present. KLEBS believes that in these cases the development of the roots is due to the increased absorption of water, and holds this factor to be of great importance in other regeneration phenomena. The following experiments will throw light upon this.

The question was attacked by two opposite methods: the effect was determined of (1) growing plants without injury or any removal under such conditions as would increase the water in the plant to the highest amount possible; (2) of supplying the other conditions for regeneration and at the same time decreasing the water content of the plant to the least possible amount compatible with actual existence.

Experiment 9.—Six plants in pots were placed under bell jars, whose inner surfaces were lined with wet filter paper. The plants grew rapidly, but no regeneration occurred.

Experiment 10.—The same experiment was repeated with five other plants, with the same result. While this usual method of forming a moist chamber gives an atmosphere that minimizes transpiration, it does not altogether inhibit it. This objection was overcome in two ways (experiments 11 and 17).

Experiment 11.—Two plants were set in a large bell jar, and this was set over a vessel of water so that the bottom of the bell jar was just below the surface of the water. This water was heated to and kept at a constant temperature of 33°. The air of the greenhouse averaged 18–20°, while that in the bell jar was 24°, a good growing temperature for this plant. The air surrounding the plant was thus saturated from a water surface whose temperature was 7° higher than the temperature of the plant, which entirely inhibits any evaporation from the latter. The plants grew rapidly, but the primordia at the base showed no signs of developing. Here the plant is undoubtedly saturated with water.

Experiment 12.—Attempts were made to prevent transpiration by covering the leaves with vaseline, cocoa butter, or soft wax, but in no case was the development of the buds produced.

Experiment 13.—In the moist chambers described the whole plant was in the moist air. Two plants were next arranged, each in a glass

cylinder, so that the lower part of the stem of each plant passed through a rubber stopper inserted in the bottom of the cylinder. Thus the stem and foliage were in the cylinder, the cotyledons were below in the air, and the roots hung down into a nutrient salt solution. The cylinder was lined with wet filter paper and covered at the top. The plants grew rapidly, but the basal primordia did not develop.

Experiment 14.—Three other plants were arranged in the same way, except that the cylinders were filled with water instead of moist air. The stems grew rapidly for a few days, then the growth gradually decreased, and finally ceased entirely. No development of the basal buds occurred until growth ceased, when they developed. This was repeated by inverting three plants with their foliage in a large aquarium, with the same result. When the shoots were killed by the prolonged submergence, however, the basal buds developed shoots.

Experiment 15.—All the foliage was cut from five plants, thus removing the transpiration surface, and no development of basal buds followed. All the leaves and also the cotyledons were removed from three other plants, with the same result.

Experiment 16.—Five large plants, with six to eight internodes and a large display of foliage were selected. The vegetative tip was removed from each and also the buds in the axils of all the leaves. In four of them the basal buds developed. Here the disturbance in the water content could only have been trifling, for careful determination before and after cutting off the tips showed no appreciable diminution in the amount of transpiration.

Experiment 17.—A second moist chamber that kept the plant saturated was formed in the following way: a glass tube was drawn to a fine end with a capillary opening and through this a fine jet of water was forced, which struck a small piece of ground glass held obliquely to it and was scattered into a fine mist. In this mist five plants were set, the fine spray continuously settling on every part of the plant, not only the upper but also the under surface, for it circulated freely on the slight air currents always present. Here the plants were covered constantly with a thin layer of water, which completely checked transpiration, but the excellent aeration allowed a healthy growth. All the plants grew rapidly, but there was no development of the buds at the base.

The experiments thus far show that the maximum amount of water in the plant will not induce the basal primordia to develop. The opposite method was then tried, *i. e.*, supplying the other possible factors in regeneration and at the same time withdrawing water from the plant.

Experiment 18.—Eight plants were used, the soil allowed to dry until the leaves wilted, and the stems then cut off a few centimeters above the ground. No more water was added and the soil became quite dry. The remaining part of the stems wilted and the cotyledons began to shrivel. In spite of this the primordia developed on six of the plants; on the other two they started but soon withered completely. The six that developed grew very slowly and apparently suffered severely from lack of water. Three of them were then watered, and at once recovered turgidity and grew normally. On the other three the young shoots reached an average length of 3^{cm} and then died. Here the buds started to develop with a turgidity much below the normal, and continued slowly against a decreasing turgescence until the death point was reached. This part of the experiment was repeated on several occasions and always with the same result. Check plants under the same conditions, but with the stems not removed, showed no development of the buds. The following more accurate method was then used.

Experiment 19.—To the weak nutrient salt solution in which the plants were frequently grown was added KNO_3 to make up solutions of the following gram-equivalent proportions: $\frac{2}{3}$, $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{4}$, $\frac{1}{5}$, $\frac{1}{6}$, $\frac{1}{7}$, $\frac{1}{8}$, $\frac{1}{9}$, $\frac{1}{10}$. To each of these were transferred plants whose roots had been grown in the usual nutrient salt solution. In four days the roots in the $\frac{2}{3}$, $\frac{1}{2}$, and $\frac{1}{3}$ gram-equivalent solutions were entirely plasmolyzed and killed, and the stems and leaves wilted. In the $\frac{1}{4}$ solution the roots were partially plasmolyzed, though not killed, and the leaves were just flaccid. In the $\frac{1}{5}$ solution the roots seemed quite healthy, and also the shoots, but growth was very slow. In all the other solutions the plants were vigorous and grew rapidly. Another set of these solutions was made up, and in each were placed plants with vigorous roots that had been developed in nutrient salt solutions, and each stem was cut off. As before, those in the $\frac{2}{3}$, $\frac{1}{2}$ and $\frac{1}{3}$ solutions were killed by plasmolysis; in the $\frac{1}{4}$ solution the

roots at the end of the week were killed, but the stem was still alive, though wilted, and the buds were developing; the cotyledons were much shrunken from loss of water which had been drawn out by the strong solution. The plants lived for several weeks, the young shoots slowly developing, and then died as the solution became stronger through evaporation. In the $\frac{1}{5}$ solution the development of the buds was slow but normal. In all the others it was quite active.

Experiment 20.—The nutrient salt solution mentioned was made up in concentrations which approximate the following: $\frac{25}{100}$, $\frac{30}{100}$, $\frac{35}{100}$, $\frac{37}{100}$, $\frac{40}{100}$, $\frac{45}{100}$, $\frac{48}{100}$, $\frac{50}{100}$, $\frac{55}{100}$, $\frac{60}{100}$ gram-equivalent solution. These concentrations are only approximate, for probably ionization is not complete in any, certainly not in the stronger solutions, and also sufficient KOH was added to make them neutral. As it is only the relative strengths of solution that are required, it was not necessary to determine the actual osmotic pressure. One plant was used for each solution, and as before the roots were in the solution and the cotyledons and the rest of the plant in the air. Each plant was cut off a few centimeters above the cotyledons. In the three strongest solutions the plants were entirely killed, wilting very rapidly. In the $\frac{43}{100}$ gram-equivalent solution, after two weeks the roots were still alive but very much twisted and contorted, and so far as could be seen no growth had occurred in them. The development of the buds was slow but complete. In the next solution below this the growth of the buds was considerably faster, and in all the others it was normal. Similar plants with their shoots and leaves intact were put in the $\frac{45}{100}$ and $\frac{48}{100}$ solutions and in two days the leaves had wilted down.

Experiment 21.—The last experiment was repeated by cutting off not only the stem but also the cotyledons. The result was the same, new shoots slowly forming while the water was being withdrawn.

The result of these experiments shows (1) that the buds in the axils of the cotyledons will not develop under the influence of maximum turgidity of the cells so long as the upper part is still functioning, and (2) that if the stem be cut off the buds will develop against a partial plasmolysis of the plant. With the shoot intact no change in the water content will start the buds into activity, so that the stimulus to their development does not need to include any change in the water content of the cells. The factor that is constant in all the cases is the removal of the growing points above.

If WIESNER'S conception that checking transpiration and consequent accumulation of water is the cause of the development of buds when the leaves are removed is correct, we should expect to find the same development if we occasion an equal accumulation of water without the removal of the leaves. But this does not happen. Experiments were conducted on young shoots of Salix, Populus, Cornus, Ulmus, Solidago, Silphium, and other plants. These need not be described individually, it being sufficient to say that when grown in the moist chambers described above, including the one in which the chamber was filled perpetually with fine spray, the buds showed no tendency to develop. In some cases cuttings were used, and in others plants with roots in soil or in nutrient water cultures. No loss of water was possible anywhere, and every part of the plant capable of taking in water was doing so. On the other hand, as will be discussed later, the buds promptly develop even when very plainly suffering from lack of water, if the tip of the shoot be removed.

Roots of Taraxacum with all buds removed were left in rather damp air, but yet allowing a slow evaporation of water from the surface. While thus slowly drying they all regenerated new buds and shoots. WAKKER obtained buds on leaves of Bryophyllum when submerged, but, as GOEBEL has mentioned, we do not know what other factors come into operation in the course of such drastic treatment. One of the most striking cases of the direct influence of water in inciting regeneration is that of *Cardamine pratense*, which, as GOEBEL (3, p. 425) showed, produces shoots on the leaves while still intact, when the plant is placed in a moist chamber. Bud primordia in this case are already formed on the leaves, and in their moist shady habitat in nature, where vegetative growth is luxuriant, develop abundantly, so that here we are probably only dealing with the usual precocious vegetative growth of this plant.

It is in the case of root production that we get what at first seems to be the most striking examples of the direct influence of water on the origin of new parts. It is well known that many stems form roots when cut off and placed in water, and KLEBS, as mentioned above, has shown that in Salix the application of water to local areas of the stem, without any wounding, is followed by a copious appearance of roots. But even here a closer analysis of the condition may

reveal other factors than those which have been considered the most important. Experiments on this connection are to be mentioned. KLEBS (**5,** p. 109) says that when through wounding or separation roots or shoots either infold themselves or are produced entirely anew, it is because through this separation just those conditions are brought about in the cells which under all other circumstances would start into operation the same "building processes;" and this condition in Salix he asserts lies in the necessary accumulation of water in the parts concerned. Experiments in this connection are to be mentioned. VÖCHTING (**13**), in his classic experiments on Salix, obtained roots on pieces of stem when placed in moist air, and concluded that the cause of root development was the increased moisture. This assumes that the stems absorbed moisture from the atmosphere, and also that if they had not absorbed this the roots would not have developed.
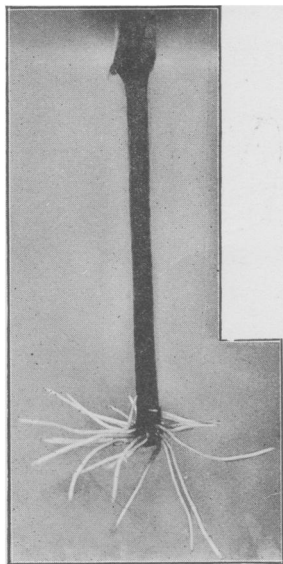

FIG. 5

Before we can attribute a result to any factor it is necessary to show (1) that that factor is always present when the result occurs, and (2) that when it is absent the result will not occur. Will roots of Salix, for example, develop on the stem only after "the necessary accumulation of water;" or can we produce them without this, or with even a decrease of water in the parts producing them? The following experiments were to determine this. For convenience, those on Phaseolus will be described first. If the stem of this plant be cut off anywhere and placed in water, roots come out abundantly at the lower end (*fig. 5*). These arise from the pericycle entirely anew, no root primordia existing anywhere on the stem.

Experiment 22.—Entire plants, with roots in soil or water cultures, were placed in the three moist chambers described above. They grew rapidly and no roots formed on the stems. There can be no doubt that the stems were entirely saturated with water.

Experiment 23.—Plants with roots grown in water culture were placed in water so that the stems were submerged. From several the cuticle and outer part of the cortex was peeled off, so that water would enter freely, and in some the foliage was enclosed in a moist chamber. No roots developed.

Experiment 24.—Stems with roots intact were submerged as in the last experiment, but some of the stems were slit through the middle longitudinally, while from others a slice out of the side the length of the internode and one-third the diameter was taken. Thus free
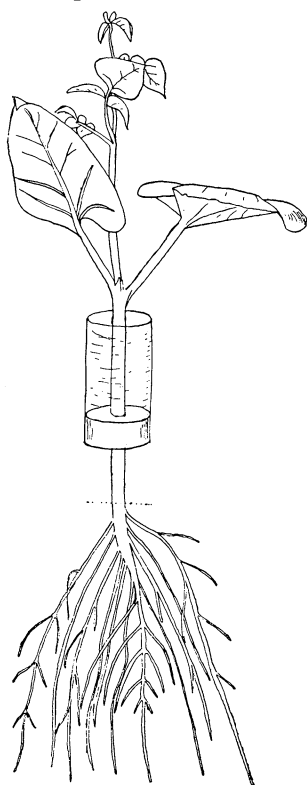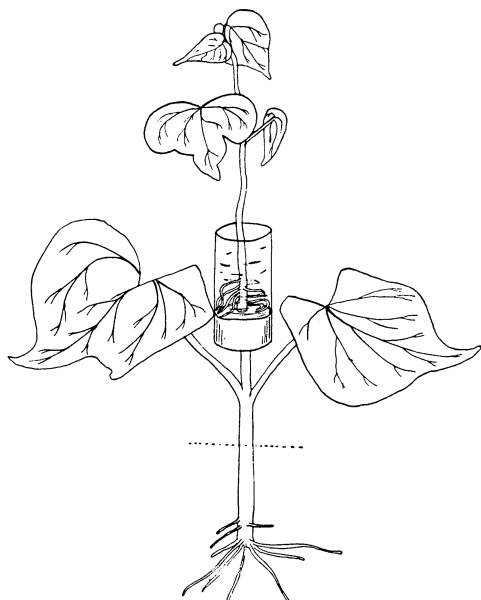
Fig. 6   Fig. 7

absorption of water was possible all along the stem, but no roots developed. If, as will be discussed again, the cut be a transverse one, severing some of the bundles, roots promptly develop just above this; or if the roots be removed new roots form along the stem.

Experiment 25.—Stems with roots intact below were surrounded at local regions by water in glass cylinders (*fig. 6*), and the cortex was removed so as to allow free absorption. No roots developed. If, however, as in *fig. 7*, stems were used from which the root

system had been removed, roots promptly came at these watered areas.

These experiments show that contact with or the free absorption of water by the stem, or the complete saturation of the stem and whole plant, will not induce root development on the stems when the roots below are intact.
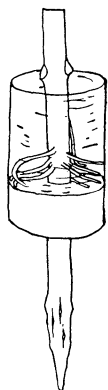

FIG. 8

On the other hand, the roots may develop when the parts from which they arise not only do not absorb any water, but are actually wilting. From a considerable number only four experiments will be mentioned.

Experiment 26.—Plants were cut off near the base and the whole plants placed in damp air, the lower free end of the stem being suspended in the air and not in contact with water. A slow transpiration necessarily occurred and the plants gradually wilted. The lower end of the stem became quite dry, yet from it roots arose.

Experiment 27.—Three pieces of internodes were placed in damp air. They wilted until there was a conspicuous shrinkage, and yet at the basal end of each roots developed. The weight of the pieces at the beginning of the experiment was 8.7^{gr}, and eight days later, when roots had just appeared, 7.9^{gr}.

Experiment 28.—Stems were cut off near the base, and a portion of the upper part of the internode was surrounded by water, as shown in *fig. 8*. The lower part projected downward through a hole in a glass plate and was in the rather dry air of the laboratory; while over all the rest of the plant was placed a bell jar to keep the air moist so that the plant would not wilt. Roots soon came out from the part of the stem surrounded by water. The basal end projecting

FIG. 9

down into the air became somewhat wilted, especially toward the base where the end for about 1^{cm} was completely dried up. In spite of this, roots formed just above this dried portion, and broke through the epidermis but could not continue in the dry air.
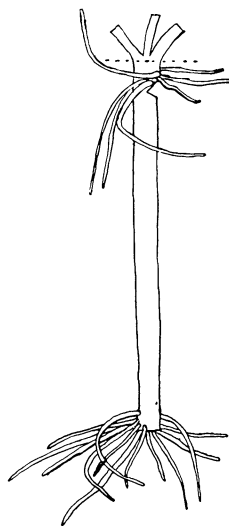
Experiment 29.—Stems were cut off and the lower part placed in water. Above the water a deep notch was cut in each stem. Roots developed abundantly at the lower end in the water, and also just above the notch. The vessels being cut off from the water supply, the tissues were quite wilted and shrunken. In the dry air the roots did not elongate more than $1-2^{mm}$, but if the air is moist they grow vigorously (*fig. 9*). If, as stated, a portion of the stem is surrounded by water, no roots appear on this part so long as the roots are intact below (*fig. 6*); but if the latter are cut off, roots appear at the former place as well as at the place below from which they were removed. But the cutting off of the root system in this case cannot influence the amount of water in the stem, unless it be to diminish it.
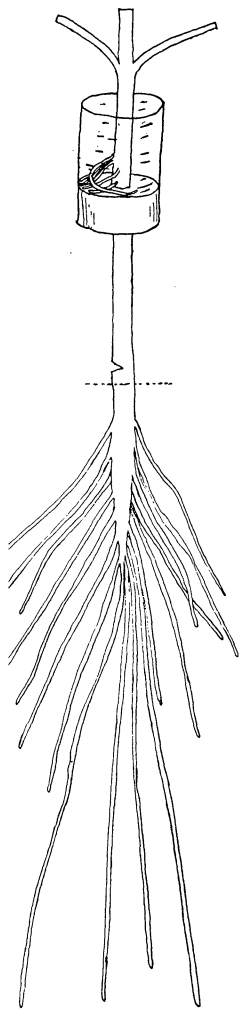
Experiment 30.—The plant was fixed as shown in *fig. 10*. Below the cylinder of water surrounding the stem a notch was cut about one-third the way through the stem. This severed the connection with the root, along these bundles, from this point upwards. Roots appeared in the water above, on the side directly above the notch, and from the bundles severed by it. Here again, if the cutting of the notch had any effect on the amount of water in the part of the stem directly above it, it would only be to diminish it, yet its effect was to produce roots there.

Experiment 31 (fig. 11).—Both portions *a* and *b* are in the water, and if there is any difference *a* has the better chance of becoming saturated; yet *b* alone produces roots.

Experiment 32 (fig. 12).—The apical end is inverted in water and the basal in somewhat damp air, but allowing considerable evaporation. No roots at all come on the former, but on the latter many primordia are formed and break through the cortex, and a few grow out into the air. If the air be kept saturated, or the end surrounded by water, many roots grow out vigorously.

FIG. 10

These experiments all show, at least in the bean, that an increase of water at any point along the stem will not in itself incite the formation of roots, and that root primordia will be organized when the cells there contain much less water than when growing normally. Their subsequent development depends on sufficient external moisture to prevent them from wilting.

In Salix, root primordia are laid down early along the stem in the vicinity of the buds. In some species, at least, contact with water, as KLEBS has shown, will incite these to active growth. It does not necessarily follow, however, that this is due to the increased absorption of
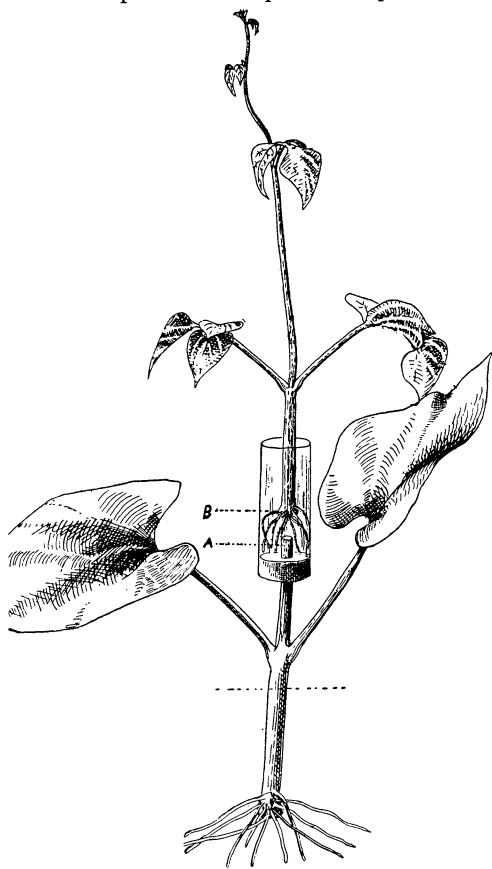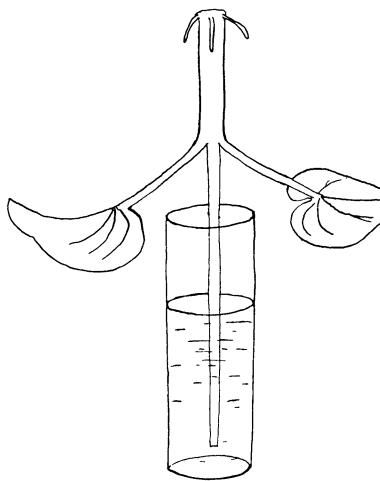

FIG. 11

FIG. 12

water by these cells, as KLEBS maintains. The following experiments throw some light on this.

Experiment 33.—Three pieces of *Salix glaucophylla* stem two years old were cut off from plants which were growing in pots in the greenhouse and weighed. The aggregate weight was 27.5^{gr}. These were placed horizontally in a chamber where the air was just moist.

In less than a week roots had appeared on all three near the base. The weight now was found to be 26^{gr}, a loss of 1.5^{gr}, most of which certainly was water. Another piece at first weighed 10.5^{gr}; and when the roots coming out on it were 1^{cm} long the weight was 10.1^{gr}. Thus, with the piece as a whole losing water, but with a moist atmosphere outside, roots develop.
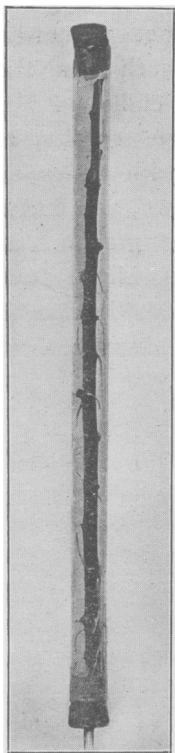

FIG. 13

Experiment 34.—Two pieces of stem 30^{cm} long were fastened with their basal ends connected with the water faucet, so that the water was forced into them under high pressure. This pressure was sufficient to cause water to ooze slowly from the opposite end of the stem. The air surrounding the stem was that of the laboratory. No roots anywhere enlarged enough to break through the cortex to the surface. Here a greatly increased amount of water in the stem does not start the roots.

Experiment 35.—Stems growing with vigorous roots in water cultures were selected. A few inches above the water a ring of bark 5^{mm} wide, cutting into the wood, was removed. Just above this for about 1^{cm} the bark (*i. e.*, all outside of the wood) was plainly drier than at any other point, yet the root primordia in this part in a few days enlarged and broke through the cortex and epidermis, but went no farther; but when one of the pieces was put in moist air they grew out rapidly.

Experiment 36.—Five pieces of stem of *Salix fragilis*, each 30^{cm} long and one and two years old, were cut off from larger branches growing with roots in water. Two of the pieces bore young leaves, while on the other three the buds had not yet opened. Each piece, carefully freed from any water on the surface, was placed inside a dry glass tube as small in diameter as would admit the piece (*fig. 13*). Each tube was sealed air tight at each end by a rubber stopper and wax. Here there was no possibility of any absorption of moisture; on the other hand evaporation was constantly going on from the surface of the stems, and the moisture condensed in little droplets on

the inside of the tube.   The air in the tube under these circumstances must have been saturated with moisture, all of course at the expense of the water in the stem.   Within a week three of the stems and a few days later a fourth showed vigorous roots coming out, which grew rapidly and soon were several centimeters long.   Here again the stimulus certainly was not any in-
creased water in the stems.   It seems
as though a moist atmosphere outside
of the stem can act as a stimulus with-
out any increase, in fact even a de-
crease, inside.   How this could act
through the epidermal and outer corky
layers is not clear.   At first it seemed
that the real cause lay in the removal
of the piece from all influence of the
roots below, but glass tubes similarly
placed around portions of longer pieces
whose roots were intact and active
below resulted in the production of

FIG. 14

roots just the same.   Similar pieces covered with a thin coat of wax to prevent any evaporation showed no signs of root development.

In submerged aquatics, where there is no current of water through the plant, but where the absence of a cutinized epidermis allows free diffusion in and out at every point, and where all the cells are constantly saturated, the removal of a part of the stem does not cause any change in the amount of water present.   If such plants regenerate it is not due to disturbances in the water content.

Experiment 37.—Portions of the stems of the extreme aquatic forms of *Proserpinaca palustris* and *Ranunculus multifidus* were severed from the parent plants and left submerged.   In all cases new roots were at once organized and grew rapidly at or near the basal end, and at the other end shoots started from the latent buds in the leaf axils (*fig. 14*).   Isolated pieces of roots of Taraxacum, *Rumex crispus*, and stems of Zamia all organized new shoots while they were still losing moisture.   In cases like Salix there is no doubt that contact with water will start the development of roots along the stem, yet these can also be started by other causes while the cells are losing

water. In the great majority of instances where regeneration occurs, however, it cannot be due to any disturbance in the amount of water present in the parts concerned. Several leaves of Begonia and Bryophyllum were kept in the air of the room, but with their petioles in water. The blades quite plainly were not more turgid than those left on the plants, and not so much so as those on plants grown in moist air; yet they produced buds while the latter did not.

The results of experiments with the other factors mentioned will be presented in the second paper of this series.

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